REMARKS/ARGUMENTS

There are now 17 claims pending.

Rejection under 35 USC §102(b)

Claims 1, 6, 9 to 10, 13 to 14, 16 and 20 of record remain rejected as anticipated by Burchi *et al.* The Examiner states that Applicant's previous argument was unpersuasive and maintains that all of the limitations of the rejected claims are taught by Burchi *et al.*

Former claim 1 has been amended to specify in step (b) that *a root of the dicot plant is suspended in buffer*. Support for the amendment to claim 1 may be found, for example, in the description at page 6, lines 24 to 27 and in Figure 1. This amendment has rendered the subject matter of claims 14 and 15 redundant which claims have therefore been cancelled.

Further, former claims 16 and 20 have also been amended to better distinguish the claimed subject matter from the prior art by specifying that the transgenic plant produced by the method of claim 1 or 8 is *stably transformed*. Burchi *et al.* only teach transient expression of a transgene using their transformation method and thus, did not demonstrate that a transgene was stably introduced into the plant DNA and inherited by the T1, T2, T3 and T4 progeny plants. Further, as the study focuses on ornamental plants, Burchi *et al.* do not seek to develop a stable transformation technique that relies on cross-breeding of plants because they indicate that efficient systems of sexual reproduction in most ornamental species cannot be relied upon. This is apparently due to sterility or incompatibility problems associated with this species of plant and also, commercial quality does not depend on measurable traits. Support for the amendment to claims 16 and 20 may be found, for example, in the description at page 3, lines 26 to 29, page 5, lines 1 to 5 and in the "Results" section spanning page 12, line 20 to page 13, line 23.

Further, in light of the Examiner's comments that the transgenic plants of claims 16 and 20 are indistinguishable, Applicant respectfully disagrees. The method of claim 8 (on which claim 20 depends) differs in scope from the method of claim 1 (on which claim 16 depends) in that the method recites the additional feature that the plasmid vector comprises a gene for barley oxalic acid oxidase. Therefore, the transgenic plant defined in claim 20 also contains this same gene and is therefore distinguishable from the plant produced by the method of claim 16. The Examiner is respectfully reminded that under U.S. patent practice, an Applicant is permitted to restate the invention in a reasonable number of ways by plural claiming. A mere difference in scope between the claims has been held to be enough. (See, for example, MPEP 706.03(k))

Burchi *et al.* disclose preliminary results of transient GUS gene expression in axillary shoots of carnation, chrysanthemum and lisianthus transformed *in vivo* by the electrotransfection method (see page 164, 1st column, 1st full paragraph). After the test plants were grown in pots to an appropriate stage of development, a platinum wire connected to the anode was inserted into the soil in contact with the roots (see page 164, 1st column, last paragraph). As reported therein, only

about 50% of the treated plants survived and about 50% of those plants which survived showed transient GUS expression. The poor survival rate of the plants has been attributed primarily to mineral toxicity effects caused by an increased uptake of positively charged ions from the soil to the roots of the plant following electrophoresis; the positive ions presumably concentrated at the apex of the plant. This was despite the fact that fertilization of the plants was stopped several weeks before electrophoretic transfection to purposely avoid mineral toxicity effects.

In light of the foregoing, Applicant submits that Burchi *et al.* do not set forth each and every element as defined in claim 1, as amended (and dependent claims thereto) and therefore, do not anticipate the claimed subject matter. Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

Rejection under 35 USC §103(a)

Claims 3, 5, 7 to 8, 11 to 12, 15 and 21 to 24 are rejected as obvious over Burchi *et al.* in view of Bidney *et al.* The Examiner states:

> "Although Bidet [sic] *et al.* do not specifically teach a method wherein an *Agrobacterium* plamid vector containing a transgene is electrophoresed into a host plant, they do teach a combination particle bombardment followed by the use of *Agrobacterium* for DNA delivery. One of ordinary skill in the art would have been motivated to substitute *Agrobacterium* binary vectors containing a transgene for the plasmid used by Burchi *et al.* given that it is well known in the art that transformation of dicots is enhanced using *Agrobacterium*..." [Emphasis added.]

Applicant respectfully disagrees.

Firstly, in order to establish a *prima facie* case of obviousness, the prior art references(s) must teach or suggest all of the elements and limitations recited in the claims.

Burchi *et al.* teach a DNA transfer method into the intact meristem of adult plants grown in pots under controlled conditions using electrophoresis. The plants were cultivated to promote the development of axillary buds close to the soil so as to reduce the distance between the cathode and anode of the power supply which in turn affects the electrical resistance and amount of voltage and/or running time to achieve adequate DNA migration. A platinum wire of the cathode was placed on the exposed meristem dome of an axillary shoot and a second platinum wire was inserted into the soil close in contact with the roots of the plant.

On the other hand, Bidney *et al.* disclose a method of producing a pathogen resistant hybrid plant using explants[1] and a combination of wounding plant tissue by particle bombardment, followed by co-cultivation of the explant with *Agrobacterium* to facilitate DNA delivery. The explants were subjected to microprojectile bombardment (column 20, lines 31 to 41) prior to *Agrobacterium* treatment (column 20, line 56 to column 21, line 25). The soybean transgenics,

1 An explant refers to a piece of tissue of a donor plant (e.g. leaf, stem section, apical meristem) that is excised and transferred to tissue culture media.

to which the Examiner refers (column 36, Example 3), were produced in accordance with the protocol described in U.S. Patent No. 5,563,055 (hereinafter referred to as the '055 patent).

The protocol described in the '055 patent involves the co-culture of soybean explants with *Agrobacterium* species carrying a plasmid into which is inserted the gene of interest. *Agrobacterium*-mediated transformation involves the co-cultivation of the explant with an *Agrobacterium* species having a "binary" tumor-inducing (Ti) plasmid vector system comprising: (1) a Ti-plasmid which carries a single-stranded copy (T-strand) of the bacterial tumor-inducing DNA (T-DNA) (and into which is inserted the gene or genes of interest); and (2) a "helper" plasmid that encodes specific *Agrobacterium* virulence proteins (Vir) essential for T-DNA transfer as they associate directly with the T-strand to form a transport complex (T-complex). Nuclear import of the T-complex culminates with T-DNA integration into the host genome. Since the T-DNA molecule itself does not contain specific signals for nuclear import, T-DNA insertion into the plant DNA must be mediated by proteins transported from *Agrobacterium* itself. Vir proteins of the T-complex have been implicated in the integration process. Thus, the genetic transformation is achieved by bacterial attachment to the plant cell surface, transfer of T-DNA from bacteria to plant cells across the plant wall and membrane, nuclear transport of the T-complex, and stable integration of T-DNA into the plant genome. These events involve direct interactions between plant proteins and *Agrobacterium* virulence (Vir) proteins that are exported to the plant and accompany the T-DNA on its journey through the plant cell to the nucleus.

The role of the Vir proteins in facilitating T-DNA transfer is further supported at column 2, line 50 of the '055 patent, where it states:

> Several factors which significantly impact the transformation of cultured soybean cells have been identified in arriving at this invention. The most important of these appears to be the induction of the virulence (vir) genes in Agrobacterium by proper use of signal molecules during cocultivation. Cultured soybean cells lack or have a limiting amount of the necessary signal molecules to initiate the transformation process. These results are in general agreement with other studies which have recognized the importance of vir gene induction for soybean transformation but failed to solve the problem...This invention uses acetosyringone, a phenolic compound produced by wounded plant cells, to induce the vir genes...Use of adequate amounts of signal molecules in the cocultivation process has in every instance resulted in enhanced transformation frequency. [Emphasis added.]

At column 3, line 5 of the '055 patent, it also states:

> The temperature for cocultivation was discovered to be another important factor.

At column 3, line 23 of the '055 patent, it further states:

> The successful transformation of soybean cells was also dependent upon the concentration of bacteria in the inoculum. In general, higher numbers of bacteria resulted in more transformation events.

In contrast, the instant application teaches a method of transformation using a mature intact plant or seedling, a single Ti-plasmid and electrophoresis. No where in the description or the examples is the invention described or claimed as employing (1) an explant, (2) a "binary" system that necessiates the use of a "helper" plasmid that encodes specific *Agrobacterium* virulence (Vir) proteins, (3) co-cultivation of an explant with *Agrobacterium*, and/or (4) preliminary wounding of the plant by microprojectile bombardment to further facilitate transfer of the T-DNA into the plant.

On this basis, Applicant respectfully submits that neither Burchi *et al.* nor Bidney *et al.* teach or suggest all of the elements and limitations recited in the claims.

Secondly, there is no suggestion, teaching or motivation to combine the references on which the rejection is based. Neither of the prior art references suggest any desirability to combine the elements as claimed for transforming a plant using electrophoresis and DNA comprising a plasmid vector.

Thirdly, a person of skill in the art would not have any reasonable expectation of success that the combination of Burchi *et al.* nor Bidney *et al.* would work to produce beneficial results or that a person of skill in the art should be able to arrive at a claimed invention through a minimum of experimentation. This is particularly evident in light of the comment made at column 2, line 50 of the '055 patent where it states that "cultured soybean cells lack or have a limiting amount of the necessary signal molecules to initiate the transformation process". On this basis, a skilled person would assume that the transformation process would not work without the "helper" plasmid (i.e. Vir proteins). This teaches away from the instant method, which does not rely upon the presence of Vir proteins or co-cultivation of plant cells in *Agrobacterium* in order for stably transformed plants to be produced.

Based on the combined teachings of Buchi *et al.* and Bidney *et al.*, it is therefore asserted that a skilled artisan would have absolutely no reasonable expectation of success that an intact plant could be transformed with DNA by applying a low amperage current using standard buffers as described in the instant application. In fact, based on the teaching of Bidney *et al.*, a skilled person would likely expect that without (1) the induction of the virulence (Vir) genes in *Agrobacterium* to provide the necessary signal molecules to initiate the transformation process during co-cultivation, and (2) the use of a phenolic compound produced by wounded plant cells to induce the Vir genes, the method of transformation would likely fail. Accordingly, Applicant submits there is no suggestion in the teachings of either reference or predictability in the art that would provide direction for a skilled artisan to follow in order to arrive at the claimed invention with any reasonable expectation of success.

Reconsideration and withdrawal of the rejection are respectfully requested.

Appl. No. 09/698,106

The Examiner is respectfully urged to call the undersigned at (613) 232-2486 to discuss the claims in an effort to reach a mutual agreement with respect to claim limitations in the present application which will be effective to define the patentable subject matter if the present claims are not deemed to be adequate for this purpose.

In view of the forgoing, early favorable consideration of this application is earnestly solicited.

Respectfully submitted,

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